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The effect of prey identity and substrate type on the functional response of a globally invasive crayfish

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Abstract

Biological invasions threaten biodiversity on a global scale, therefore, developing predictive methods to understand variation in ecological change conferred is essential. Trophic interaction strength underpins community dynamics, however, these interactions can be profoundly affected by abiotic context, such as substrate type. The red swamp crayfish (*Procambarus clarkii*) has successfully invaded a number of freshwater ecosystems. We experimentally derive the Functional Response (FR) (density dependent predation) of the red swamp crayfish preying upon both a benthic prey; chironomid larvae, and a pelagic prey; *Daphnia magna*, on a no substrate control, sand, and gravel substrates to determine whether (1) there is a higher impact on prey that are benthic, and (2) whether the presence of different substrate types can dampen the interaction strength. We apply and demonstrate the utility of the Functional Response Ratio (FRR) metric in unravelling differences in ecological impact not obvious from traditional FR curves. *Procambarus clarkii* is capable of constantly utilising high numbers of both benthic and pelagic prey items, showing a Type II functional response under all scenarios. The presence of gravel and sand substrate each independently decreased the magnitude FR upon *D. magna*. Though, with regards to chironomid larvae the FR curves showed no difference in magnitude FR, the FRR reveals that the highest impact is conferred when foraging on sand substrate. This reinforces the need for impact assessments to be contextually relevant.

Keywords

Aquatic invasions, functional response ratio, invader impact, macroinvertebrates, *Procambarus clarkii*, substrate

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Introduction

Aquatic biological invasions are increasing at an alarming rate driven by increased connectivity due to new trade routes and ongoing climatic change (Davis and Darling 2017; Carlton and Fowler 2018; Seebens et al. 2017; Meyerson et al. 2019). Such invasions can result in substantial loss of biodiversity and homogenisation of communities (Bellard et al. 2016; Courchamp et al. 2017), which is often quantified and defined as "ecological impact" (Ricciardi et al. 2013; Blackburn et al. 2014; Dick et al. 2017a, b). The Aichi Biodiversity Targets (SCBD 2010) emphasise the importance of developing affirmative action to reduce biodiversity loss in order to increase resilience of ecosystem service production by reducing threats. Therefore, predicting and assessing the possible damage caused by invasive species is imperative to conserving biodiversity and valuable services derived from freshwater systems (Dickey et al. 2018; Cuthbert et al. 2019). Risk assessment and impact evaluation previously relied heavily upon invasion history (Ricciardi and Cohen 2007), which is of course of no use for emerging and new invasions. Recently, there has thus been an emphasis on quantifying and predicting invader impact before invasion occurs or in the initial stages of an emerging invasion (Dick et al. 2014, 2017a, b; Dickey et al. 2018). By determining impact under different environmental contexts, both mechanistically and phenomenologically, it is possible to identify the drivers of ecological impact exerted by a particular species on resources and incorporate preventative or mitigation measures into management policies (Wasserman et al. 2016; South et al. 2017; Dick et al. 2017a, b).

Invasive species are often characterised by their efficiency of resource consumption, whereby they generally show a higher *per capita* effect towards a focal resource in comparison to a native analogue (Dick et al. 2014, 2017a, b; Laverty et al. 2017). Comparative functional response analysis has thus been successfully employed to infer ecological impact upon resource populations across taxonomic and trophic groups (Alexander et al. 2014; Dick et al. 2017a, b; Kemp and Aldridge 2018). The functional response (FR) describes resource utilisation with respect to resource density, whereupon three categorical FR Types may be classified (Solomon 1949; Holling 1959). Type I FR, referring to a linear response is predominantly found in filter feeders as they are not limited by handling times (MacNeil et al. 1997; Jeschke et al. 2004). Type II FR describes high resource consumption at low resource density, and magnitude of the curve asymptote has been positively correlated with invader impact (Alexander et al. 2014; Dick et al. 2017b). Type III FR is a sigmoidal response that results in low consumption at low prey densities and which provides a low density refugia to prey species, and this FR type is often related to environmental stressors, aswell as prey switching or learning (Holling 1959; Murdoch 1969; South et al. 2018). Upon determining FR type, it is possible to estimate parameter values for attack rate (a), handling time (h) and maximum feeding estimates ($1/h$). Consequently, this approach has been adopted as a standardised method to quantify and compare predator (and other consumer) impact upon recipient prey (and other resource) species (Dick et al. 2017a). Further,

due to ease of calculation through laboratory experiments and even field surveys, it is possible to factorially incorporate increased complexity such as habitat, temperature, and higher order predators in order to create more realistic environmental context (Barrios-O'Neill et al. 2015; South et al. 2018; Cuthbert et al. 2018). More recently, progress into developing the FR approach towards predictive capacity has resulted in the combination of the attack and handling parameters to derive a "Functional Response Ratio" (FRR), in order to elucidate potential intermediate impact where previously distinguishing differences between FR curves were problematic (Cuthbert et al. 2019). This is centred in the assertion that high attack rate values indicate high impact, while low handling time also implies high impact, and thus the parameters are combined to create a ratio of a/h, higher values of which are found in high-impact invaders (Cuthbert et al. 2019).

Species usually form habitat associations depending on their specific life history needs or due to optimal foraging theory, wherein the most productive habitat is selected (Schoener 1971). Due to the nature of biological invasions, the abiotic and biotic features of the new range may not be analogous to that of the native range, and therefore it is imperative to assess performance and ecological impact across a number of different contexts. Benthic substrate is highly variable depending on the system and is known to provide benefits and costs to both predator and prey depending on species specific traits and fractal dimensions (Tokeshi and Arakaki 2012; Barrios-O'Neill et al. 2015). Substrate has thus been shown to dampen interaction strength, but this may be a variable response depending on the type of substrate and the foraging behaviours of the predator (Barrios-O'Neill et al. 2015; Keyler et al. 2019; Gebauer et al. 2019). Determining whether abiotic context, such as substrate type, can affect the ability of an invasive species to consume resources is integral to informing management initiatives in order to deter and reduce spread and ecological impact of non-native species.

The red swamp crayfish, *Procambarus clarkii* (Girard, 1852), is a widespread invasive species due to its wide thermal tolerance, high trait plasticity ,and popularity in aquaculture and the pet trade (Souty-Grosset et al. 2016). *Procambarus clarkii* is a polytrophic, omnivorous species which can be a destructive influence upon benthic invertebrate communities, which can result in trophic cascades and bentho-pelagic resource decoupling (Larson et al. 2017; Bucciarelli et al. 2019). *Procambarus clarkii* is spreading at an alarming rate through many countries, despite eradication and biocontrol attempts (Aquiloni et al. 2005; Nunes et al. 2017a, b; Loureiro et al. 2018). It is imperative to determine whether there is a differential ecological impact conferred by *P. clarkii* under different substrate conditions and whether this may lead to differential utilisation of prey species. Surprisingly, despite the damaging effect of *P. clarkii* on recipient systems, there is little empirical data quantifying and comparing resource consumption of important prey species. As such, we looked to address this by investigating whether there was a difference in the rate of resource utilisation over time by *P. clarkii* preying upon a benthic prey (chironomid larvae) and a pelagic prey (*Daphnia magna*) under three different substrate types (no substrate, sand, and gravel). As *P. clarkii* is a benthic species, we hypothesised that size of the prey and location in the water column

would affect the functional response magnitude and perception of the prey, wherein small pelagic prey items (i.e. *D. magna*) would be utilised less than larger benthic prey items, but that the interaction strength between *P. clarkii* and chironomid larvae would be dampened by the presence of substrate.

Methods

Specimens of sub-adult *P. clarkii* (mean ± SD: 21.62 ± 2.6 mm carapace length, n = 25) were supplied by Seahorse Aquariums (Republic of Ireland) and maintained at the Queen's University Marine Laboratory in a continuously aerated 600 L holding tank, with shelters made of drainpipe halves, supplied with dechlorinated tap water held at 23 ± 1.0 °C using aquarium heaters and subject to natural light regime. During the experimental period there was no cannibalism. *Procambarus clarkii* were maintained on commercial sinking fish food (JBL, Germany) to avoid conditioning to experimental prey items. As this species breeds at small sizes and because they are controlled species within the United Kingdom and Northern Ireland non-reproductive sub-adult crayfish were used in this experiment. Two live prey species, chironomid larvae (3.0 ± 0.6 SD mm total length) (Chironomidae) and *Daphnia magna* (0.3 ± 0.01 SD mm) (*Daphnia magna*; Daphniidae), were supplied, also from Seahorse Aquariums. Appropriate ethical approval for the use of these animals in research was obtained from the QUB Ethics Committee (School of Biological Sciences) and National Research Foundation – South African Institute for Aquatic Biodiversity (NRF–SAIAB ethics boards (25/4/1/5_2018-06).

Functional Response Experiment

A full factorial experimental design was employed to test differences in FR with regards to factors: "prey species" (2 levels), "density" (9 levels), and "substrate" (3 levels). Experimental arenas (W: 20 × L: 30 × H: 14 cm, 8 L) were held at 23 ± 1.0 °C and experiments were completed in a fully randomised design. Three substrate treatments were supplied: no substrate, commercial aquarium sand supplied at a depth of 4 mm in the experimental arena, and gravel (grain size: 8 × 4 mm) positioned using one 4 mm layer at the bottom of the experimental arena. Arenas were covered from the top and sides to avoid any synergistic or antagonistic conspecific effects. Individual predators were added per arena and allowed to acclimate for one hour before adding prey items. Each prey species was supplied at 9 densities (2, 7, 15, 40, 60, 90, 120, 200, 300 individuals per arena, n = 5 for each treatment), crayfish were allowed to feed for 1 h, after which the predators were removed, and number of prey items consumed were enumerated.

Control trials were carried out in experimental tanks at identical environmental conditions, wherein each prey species was supplied at the highest density in the ab-

sence of predators (n = 3 per substrate treatment) to determine potential background mortality. Each crayfish was re-used multiple times, but experienced each density of each prey type only once. Crayfish were given at least 3 days between use and were not fed for 24 h prior to experimental procedure to standardise hunger levels.

All analyses were undertaken using the R Statistical Software (v. 3.4.3). A generalised linear model (GLM) with a quasi-Poisson error distribution was used to determine differences in prey consumption with regards to factors "prey species", "prey density", and "substrate complexity", using a Type 3 Anova and χ^2 to report the effect size of a factor on the dependent variable. Tukey's HSD was used to generate pairwise estimates with Holm-Bonferroni adjustment of P values post-hoc.

The R package 'frair' (Pritchard 2014) was used to model the functional response type following methodology in Pritchard et al. (2017) (*frair::frair_fit*). Therein, a logistic regression was initially used to ascertain whether the proportion of prey consumed decreases with increasing prey density and consequently produces a significantly negative first order term, indicating a Type II FR. If a significantly positive first order term is obtained this indicates a Type III FR (Juliano 2001). Functional responses were modelled using maximum likelihood estimation (MLE; Bolker 2010) and Rogers' (1972) Random Predator Equation to account for prey depletion over time:

$$N_e = N_o\left(1-\exp\left(a\left(N_e h - T\right)\right)\right) \quad \textbf{(1)}$$

wherein, N_e represents the number of prey eaten, N_0 is the initial density of prey, a is the attack parameter, h is the handling parameter, and T is the total time available. The Lambert W function was implemented to fit the model to the data (Bolker 2008). The data were non-parametrically bootstrapped (*frair:: frair_boot*; n = 2000) to produce 95% BCa confidence intervals around the mean functional response curve for each treatment, wherein any overlap of BCa curves indicates a lack of statistical difference in FR. This method allows visual assessment of differences between FR curves and allows inferences to be made at a population level. Maximum feeding estimates ($1/h$) were calculated from the handling time estimates.

The FRR (Cuthbert et al. 2019) was calculated for each prey species and substrate type using the parameter estimates of a and h from the maximum likelihood estimate results of **eqn (1)**:

$$\mathrm{FFR} = a / h \quad \textbf{(2)}$$

where a is the attack parameter and h is the handling parameter derived from the FR curve. In this case the FRR is used as a diagnostic tool to determine whether there were differences that can not be observed from the usual FR outputs. Therefore, the higher the FRR value the higher the inferred impact (Cuthbert et al. 2019). As FRR results are derived from the FR values of one set of experiments and the relatively small sample size, they are, in this instance, not bootstrapped for comparison as the FR model already incorporates n = 2000 bootstrapping of parameters.

Results

Prey survival was >99% under all control treatments and thus any mortality was due to predation by *P. clarkii*, which was also frequently observed. *Procambarus clarkii* were observed in pilot experiments to feed upon chrinomid larvae by searching with their antennae and maxillipeds, contrastingly they fed upon *D. magna* in a filter feeding fashion by generating a flow via tail flicking behaviours.

All model terms significantly affected prey consumption by *P. clarkii* (Table 1). There was a significant three way interaction between 'prey × prey density × substrate' (Table 1) where at higher densities (above 120) *P. clarkii* consumed more chironomids than *D. magna* on gravel than on sand and no substrate (both $p < 0.001$) but there was no difference in prey consumption between prey species consumption on no substrate and sand ($p = 0.47$). At prey densities of 200 and above *P. clarkii* consumed more chironomids than *D. magna* on gravel compared to no substrate and sand (both $p < 0.001$), however, when on no substrate and sand *P. clarkii* consumed more *D. magna* than chironomid larvae ($p < 0.001$). Prey density and substrate significantly interaction on number of prey eaten (Table 1), whereby at higher prey densities (above 120) there were less prey consumed on gravel than on no substrate and sand (all $p < 0.001$;), however, there was no difference between no substrate and sand at densities of 120 ($p = 0.93$). Although at densities of 200 and 300 there were less prey consumed on sand than on no substrate (all $p<0.001$). Substrate type significantly interacted with prey species to affect number of prey consumed (Table 1) where there was no difference in number of chironomid larvae consumed between substrate type (all $p > 0.05$), but there were more *D. magna* consumed on no substrate and sand compared to on gravel (both $p < 0.001$) and more consumed on no substrate compared to sand ($p < 0.001$). Prey density significantly interacted with prey species (Table 1) causing more chironomids to be consumed than *D. magna* at densities of 120 ($p < 0.001$) but at densities of 200 and 300 *P. clarkii* consumed more *D. magna* than chironomid larvae. At densities below 120 there were no differences in prey consumption (all $p > 0.005$). Overall substrate type affected number of prey consumed by *P. clarkii* (Table 1) as more prey were consumed when no substrate was present compared to gravel and sand (both $p < 0.001$) and the presence of gravel substrate reduced prey consumption compared to sand ($p < 0.001$). Increasing prey density significantly affected prey consumption (Table 1) between all densities apart from between 200 and 300. Prey species significantly affected the number of prey individuals consumed (Table 1) as *P. clarkii* consumed significantly more *D. magna* than chironomid larvae ($p < 0.001$).

All prey species and substrate treatments resulted in a significant Type II FR by *P. clarkii* (Table 2, Fig. 1). The FR of *P. clarkii* towards chironomid larvae was not affected by substrate type (Table 2; Fig. 1), whereas the magnitude FR towards *D. magna* was significantly reduced by both sand and gravel substrates (Table 2; Fig.1). The attack parameter of *P. clarkii* upon *D. magna* (i.e. initial slope of the FR curve) confidence intervals overlap indicating that the attack efficiency is not driving the difference in FR

Table 1. Model terms for all factors from GLM with a quasi-Poisson error distribution used to determine differences in prey consumption with regards to factors "prey species", "density", and "substrate", using a Type 3 Anova and χ^2 to report the effect size of a factor on the dependent variable.

Model term	Chisq	df	*p*-value
Prey species	14	1	<0.001
Density	207713	8	<0.001
Substrate	78	2	<0.001
Prey species * Density	607	8	<0.001
Prey species* Substrate	112	2	<0.001
Density * Substrate	1556	16	<0.001
Prey species * Density * Substrate	1681	16	<0.001

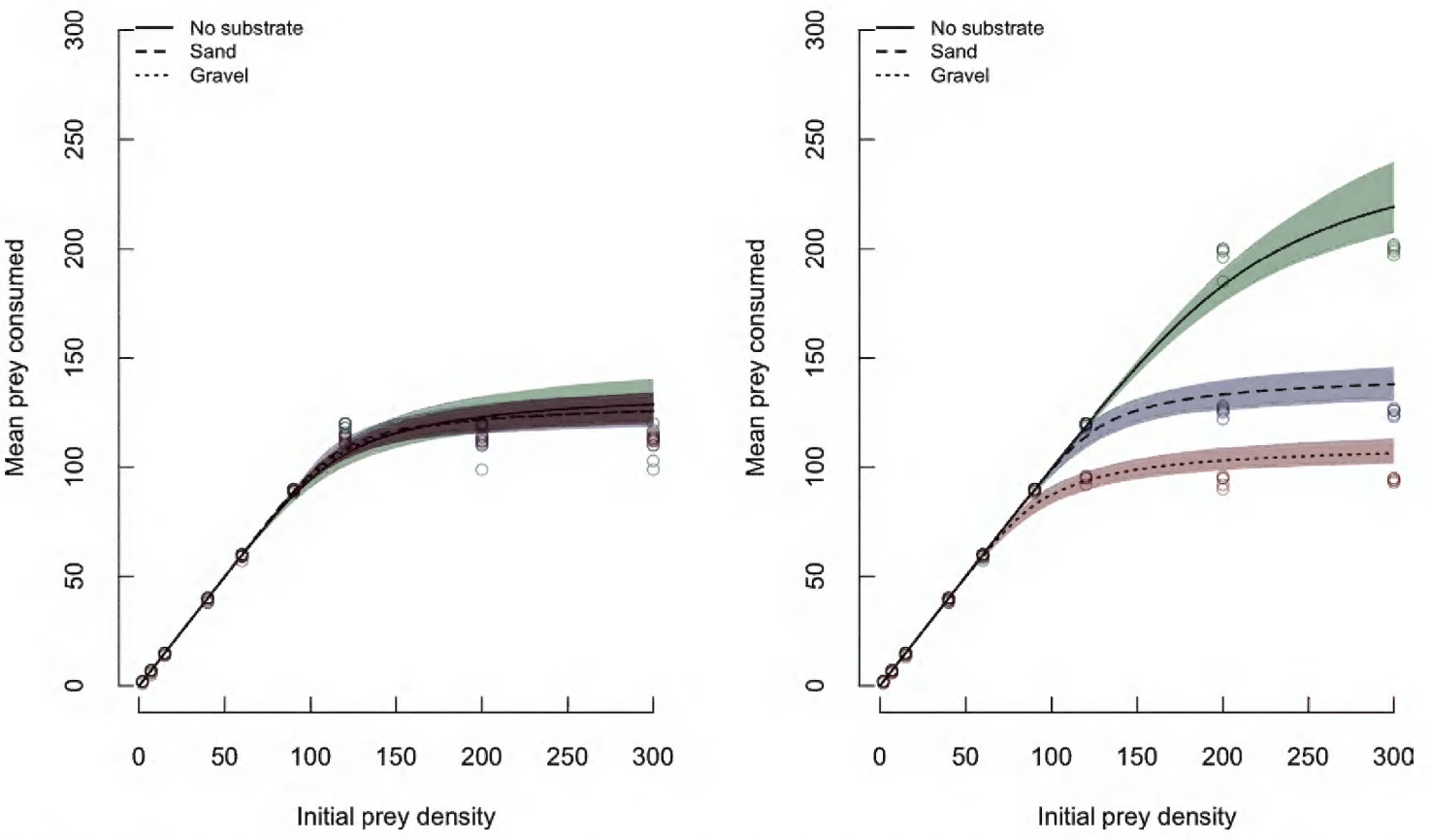

Figure 1. Functional responses of *Procambarus clarkii* preying on chironomid larvae and *Daphnia magna* under different substrate treatments; no substrate (solid line), sand (dashed line), gravel (dotted line). Points indicate raw data distributions; no substrate (green), sand (blue), gravel (brown). Shaded areas are bootstrapped (n = 2000) 95% confidence intervals.

(Table 2; Fig. 1). However, there are significant differences in the handling and maximum feeding parameters between the three substrate types, whereupon no substrate elicited the highest maximum feeding estimate, with sand intermediate and gravel producing the longest handling time and thus lowest maximum feeding estimate (Table 2; Fig. 1).

The FRR shows that when predating on chironomid larvae the impact of *P. clarkii* is highest on sand substrate compared to gravel or no substrate (Fig. 2). Whereas, when predating on *D. magna*, *P. clarkii* were almost as effective on sand and no substrate, while gravel elicited the lowest FRR for *D. magna* (Fig. 2).

Table 2. First order terms and significance levels from logistic regression of the proportion of prey consumed against initial prey density, with FR Type, functional parameters (*a*, *h*, and 1/*h*), associated significance levels from Rogers' random predator equation, bias accelerated and corrected 95% confidence intervals for *a* and *h*, and the functional response ratio (*a*/*h*) with regards to *Procambarus clarkii* predating upon chironomid larvae and *Daphnia magna* under different substrate types. *a* = attack rate; *h* = handling time; 1/*h* = maximum feeding estimate.

Prey	Substrate	First order term (*p*-value)	FR type	*a* (*p*-value)	*a* 95% BCa CI	*h* (*p*-value)	*h* 95% BCa CI	Maximum Feeding Estimate (1/*h*)	Functional Response Ratio (*a*/*h*)
Chironomid larvae	None	-0.016, <0.001	II	9.51, <0.001	6.79 – 3.53	0.007, <0.001	0.007–0.008	136.9	3102.7
	Sand	-0.016, <0.001	II	14.54, <0.001	9.30 – 28.8	0.007, <0.001	0.007–0.008	129.8	1888.3
	Gravel	-0.016, <0.001	II	12.40, <0001	8.79 – 19.08	0.007, <0.001	0.007–0.008	131.5	1631.5
D. magna	None	-0.022, <0.001	II	8.37, <0.001	6.43 – 12.09	0.003, <0.001	0.003–0.004	263.1	2202.6
	Sand	-0.017, <0.001	II	14.50, <0.001	8.60 – 28.58	0.006, <0.001	0.006–0.007	144.9	2101.4
	Gravel	-0.014, <0.001	II	9.63, <0.001	6.58 – 15.31	0.009, <0.001	0.008–0.009	111.1	1070.9

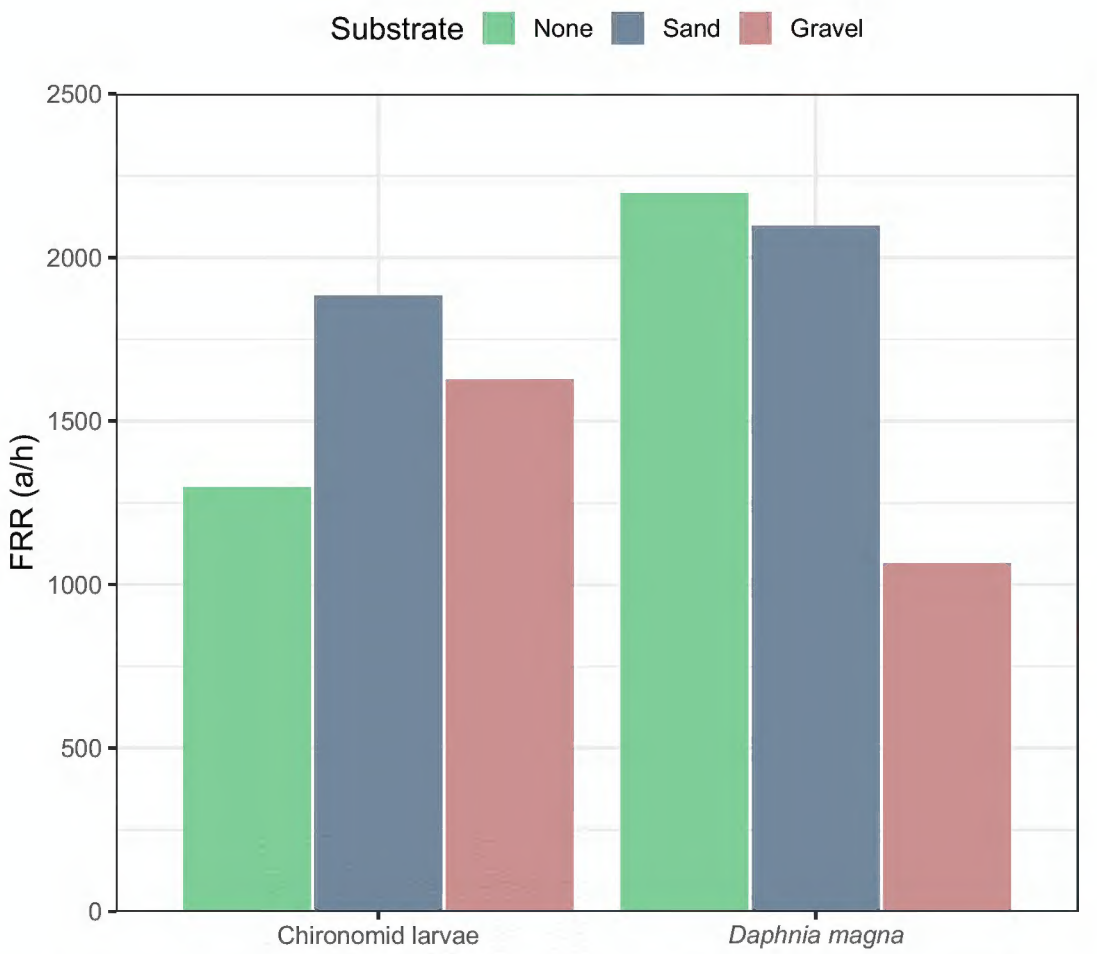

Figure 2. Functional response ratio (FRR) (*a*/*h*) of *Procambarus clarkii* preying on chironomid larvae and *Daphnia magna* under different substrate treatments; no substrate (green), sand (blue), gravel (brown).

Discussion

Management of invasive species depends upon generating contextually relevant and accurate estimates of potential impact conveyed upon a recipient ecosystem (Dick et al. 2017a, b; Evangelista et al. 2019). *Procambarus clarkii* has established as an invasive species around the globe due to human mediated transportation revolving around the aquaculture and the pet trade (Chucholl 2013; Patoka et al. 2014; Oficialdegui et al. 2019). *Procambarus clarkii* is known to alter aquatic communities through both con-

sumptive and non-consumptive effects (Carreira et al. 2017; Bucciarelli et al. 2019) but there are few quantitative estimates of their ecological impact upon macroinvertebrate communities (but see Klose and Cooper 2012).

Through the comparison of FR curves, it was possible to parameterise attack rate and handling time of a consumer upon a resource. Further, by comparing the FR curves for each prey species, we were able to distinguish whether one species may have higher pressures exerted upon it by an invasive species. Our results corroborate that *P. clarkii* indeed utilises both benthic and pelagic resources (Brown et al. 1992, 1998; Geiger et al. 2004; Lodge et al. 2005), but that the extent can be altered by substrate type but also effects prey morphology or behaviour increasing handling time. *Procambarus clarkii* has the capacity to exert substantial predatory pressure on macroinvertebrate populations as they are able to consume over 100 prey items per hour irrespective of substrate or prey identity. When taking into account possible differences in predator abundance on different benthic substrates, there could be the potential for a large per capital offtake rate of zookplankton; however, this would be very system dependent as water depth and flow dynamics affect encounter rate (Ruokonen et al. 2012). Therefore, as our experimental setup was rather shallow and devoid of natural hydrological characteristics, the results should be interpreted relative to each other rather than as a direct estimation of consumption in a system such as a large lake or stream. We recommend that habitat associations and abundance data for *P. clarkii* in invaded systems is reported on in the future in order to improve impact prediction. We also show the utility of the FRR method in determining subtle differences between impact predictions where comparison of standard FR curves become inconclusive.

The presence of different substrata affects the predation efficiency of crayfish towards each prey type. Compared to the control treatment of no substrate, both sand, and gravel substrate caused a slight reduction in the attack parameter (or search efficiency) of *P. clarkii* upon the benthic chironomid prey rather than altering the handling time. This suggests that the presence of both sand and gravel can offer a refuge for benthic prey species as the crayfish is not able to access the meiofauna as readily. Similar trends are seen in benthic invasive gobiids, where gravel substrate reduces magnitude FR compared to sand substrates (Gebauer et al. 2019). When only presented with the standard FR curve it is practically impossible to differentiate between them due to large overlap of confidence intervals. However, when the FRR is considered, it becomes apparent that due to the higher attack rate the impact exerted on chironomids when foraging on sand is higher than both no substrate and gravel substrates, despite the identical handling estimates. With regards to in situ evidence towards our results, Klose and Cooper (2012) found that *P. clarkii* abundance was negatively associated with larval chironomid abundance in two streams, one with sand and one with gravel and cobble substrate. In a similar fashion to the present results, the stream with cobble and gravel substrate showed a weaker negative relationship between predator and chironomid abundance (Klose and Cooper 2012).

Daphnia magna is an important prey species for larval and adult crayfish (Brown et al. 1992; Meakin et al. 2009; Kozák et al. 2015). Our results substantiate this, but data on field effects of crayfish species on daphniid populations is limited. Although, it is established that crayfish are polytrophic consumers, wherein invasion can lead to the

creation of new energy pathways between benthic and pelagic resources, but that this is system and species specific (Ruokonen et al. 2012; Lipták et al. 2019). While *D. magna* is generally found in the water column and exhibits diel vertical migration, this species also forages at the sediment-water interface. This behaviour thus makes this species more acccesable to predation by benthic suspension feeders such as crayfish (Horton et al. 1979). This is where the FR and FRR approaches to impact prediction shows their value by elucidating the subtle differences in strengths of behaviours controlling foraging success (Cuthbert et al. 2019). The presence and type of substrate reduced the maximum feeding rate of *P. clarkii* on *D. magna* where gravel had the lowest maximum feeding rate due to a high handling time, despite having a higher attack parameter under no substrate. It is possible that there are substantial field effects of crayfish invasions upon daphniid populations; however, further realistic context and in situ data needs to be incorporated to address the actual extent as *D. magna* can alter anti-predator behaviour in response to cues (Langer et al. 2019). Differences in impact between substrata are likely due to foraging mode in crayfish being generally benthic rather than the filter feeding behaviour observed within these experiments. Further, while *P. clarkii* is a behaviourally plastic species they may exhibit substrate preferences, especially as they are non-obligate burrowers (Hobbs and Barr 1960; Kouba et al. 2016), which could lead to differences in abundance. Further information is necessary on habitat associations and abundances of crayfish in novel environments in order to determine relative impact potential (Dick et al. 2017b)

Crayfish were able to generate a flow wherein the prey items were drawn closer, perhaps facilitating the low handling time demonstrated and indicating that filter feeding efficiency by adults is comparable to benthic foraging and thus warrants further investigation. Plasticity in feeding mode and flexible omnivory have been identified in other crustacean invaders (Platvoet et al. 2009; Kestrup et al. 2011) and could contribute to broad invasive impact. Some austral native crayfish species do not show flexible omnivory in the same manner (Johnston et al. 2011), which supports the idea that a flexible diet is a driver of invasion success and impact in invasive crustaceans (Hänfling et al 2011). Due to a focus on agonistic behaviours, descriptions or ethograms of crayfish feeding behaviours are rare in the literature (but see Panksepp and Huber 2004) and none explicitly describe the mechanism of capturing prey in a filter feeding behaviour. Filter feeding is however documented in marine crustaceans and larval crayfish (Gerlach et al. 1976; Budd et al. 1978; Brown et al. 1992; Kim et al. 2015), indicating that this may be an opportunistic behaviour facilitated by morphological similarities.

Procambarus clarkii is a global polytrophic keystone consumer which has facilitated its spread and pervasion into numerous systems (Momot 1995; Jackson et al. 2017). Due to the nature of polytrophy and generalist foraging strategies it is essential to consider multiple resources when attempting to quantify utilisation as well as context. There is substantial field and experimental data that points towards the idea that much of *P. clarkii* predation is frequency dependent (Correia 2002; Klose and Cooper 2012; Nishijima et al. 2017). Therefore, future work should focus on quantifying the frequency dependence of predation upon a variety of food resources under different contexts, especially as Nishijima et al. (2017) found that macrophyte availability also contributes to dampening *P. clarkii* impacts on invertebrate communities.

In the context of furthering approaches to impact prediction in invasion science, we establish here how the application of FR and FRR methods can be used to powerfully predict impact where traditional assessment methods would not have identified a difference between contexts. Furthermore, we discern that there is high potential for crayfish to differentially consume both benthic and pelagic macroinvertebrate species which has implications for nutrient cycling and resource provisioning for native species. In a destructive and spreading species like *P. clarkii* it is imperative to continue to determine scenarios wherein its predatory effect can be dampened in order to implement mitigation strategies as removal of crayfish once established is unfeasible.

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